

**CARDIOVASCULAR GENOMIC MEDICINE**

# Reviews of Translational Medicine and Genomics in Cardiovascular Disease: New Disease Taxonomy and Therapeutic Implications

## Cardiomyopathies: Therapeutics Based on Molecular Phenotype

Houman Ashraffian, MA, MRCP, Hugh Watkins, MD, PhD

*Oxford, United Kingdom*

The enduring subdivision of cardiomyopathies into hypertrophic (HCM), dilated (DCM), and restrictive (RCM) categories reflects the emphasis of traditional classifications on morphology. Rapid advances in the genetic interrogation of these disorders have redefined their taxonomy and revealed potential conflicts between the old and new classifications. Hypertrophic cardiomyopathy has been redefined as a disease of perturbed sarcomere function. Dilated cardiomyopathy is a disease that results from more varied perturbations, including, but not limited to, defects of the cytoskeleton. Positional cloning and candidate gene approaches have been successful in identifying >40 disease loci, many of which have led to disease genes in HCM, DCM, RCM, and arrhythmogenic right ventricular cardiomyopathy. These findings provide mechanistic insights, permit genetic screening, and to a limited extent, facilitate prognostication. Although single gene analyses rapidly focus down to the underlying mechanistic pathways, they do not take account of all relevant variation in the human genome. Correspondingly, advances in genomics, through microarrays, have facilitated characterization of these broader downstream elements. As well as refining the taxonomic reclassification of cardiomyopathies, these genomic approaches, coupled with functional studies, have identified novel potential therapeutic targets, such as cardiac energetics, calcium handling, and apoptosis. We review the successes and pitfalls of genetic and genomic approaches to cardiomyopathy and their impact on current and future clinical care. (J Am Coll Cardiol 2007; 49:1251–64) © 2007 by the American College of Cardiology Foundation

One hundred fifty years since Mendel's breeding experiments, 50 years since the discovery of the structure of deoxyribonucleic acid by Watson and Crick, and 5 years from the publication of the human genome draft, the unprecedented pace of technologic progress has inspired awe and built up expectations that genetics and genomics will provide radical insights into disease. In inherited single gene disorders, and in the relatively simple somatic genetics of cancer, important mechanistic insights have been afforded, leading to improved diagnosis, screening, and prognosis. Genomic tools have honed stratification (e.g., by the use of array technology in leukemia, breast cancer, melanoma) (1), and some therapeutics have been personalized (e.g., gefitinib in lung cancer [2]).

Cardiovascular disease is the leading cause of illness and death worldwide, with an estimated 1 million deaths annu-

ally in the U.S. alone (~40% of all-cause mortality). Specifically, heart failure has a prevalence of ~2% with an annual U.S. mortality of ~300,000 and an annual cost of \$17 billion. Most of the burden of cardiovascular disease has complex genetic and environmental origins and is only now becoming amenable to large-scale genetic analyses. Preliminary findings are beginning to indicate the potential of genetic dissection of common cardiovascular diseases (e.g., the identification of *ALOX5AP* mutations that modify leukotriene B4 metabolism and are associated with myocardial infarction and stroke) (3). But progress sufficient to already be clinically relevant has largely been confined to the less common monogenic forms of the various cardiovascular pathologies: contributions to lipid disorders (e.g., low-density lipoprotein receptor mutations in familial hypercholesterolemia), hypertension (e.g., epithelial Na<sup>+</sup> channel, ENaC, mutations in Liddle's syndrome), cardiomyopathies, and channelopathies. In the present review, we highlight the successes and pitfalls of genomic approaches to disease-gene, susceptibility-gene, and pathway discovery using the cardiomyopathies as our model. The cardiomyopathies were the first primary cardiac disorders to be understood at the molecular level, offering the potential

From the Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford, United Kingdom. Drs. Ashraffian and Watkins have received funding from the British Heart Foundation and the Wellcome Trust Functional Genomics Initiative. Cardiovascular Genomic Medicine series is edited by Geoffrey S. Ginsburg, MD, PhD.

Manuscript received July 17, 2006; revised manuscript received October 24, 2006, accepted October 30, 2006.

**Abbreviations  
and Acronyms****ACE** = angiotensin-  
converting enzyme**ARVC** = arrhythmogenic  
right ventricular  
cardiomyopathy**DCM** = dilated  
cardiomyopathy**HCM** = hypertrophic  
cardiomyopathy**LVAD** = left ventricular  
assist device**LVH** = left ventricular  
hypertrophy**MHC** = myosin heavy chain**MLP** = muscle LIM protein**RCM** = restrictive  
cardiomyopathy**SCD** = sudden cardiac  
death**SNP** = single-nucleotide  
polymorphism

for improved treatment strategies not only for patients with cardiomyopathies but also for heart failure in general.

**Genetics: Insights Into  
the Classification and  
Mechanisms of Disease**

Cardiomyopathies are a diverse and important group of heart muscle diseases which through mechanical and/or electrical dysfunction often lead to cardiovascular morbidity and mortality. The traditional classification of cardiomyopathies, guided by the observations of morbid anatomists, as befitted their then state-of-the art technology, were restricted to morphologic-clinical correlations. Those classifications have not, however, always corresponded neatly to the rapidly emerging genetic insights; accordingly a reclassification has been proposed, predicated on the findings in mendelian single-gene disorders. As described subsequently, a genetically guided classification is far from complete. Nevertheless, when grounded in the indelible genetic etiology of disease and complemented by the wealth of clinical and accumulating genomic experience, such an approach permits multiple iterations in the classification of cardiomyopathies, which will evolve into the most useful and accurate taxonomy (4).

Linkage studies, the classic approach to identifying single-gene disorders, start with the identification of extended families exhibiting a disorder, ideally with mendelian inheritance. The identification of abundant markers, such as microsatellites and single-nucleotide polymorphisms (SNPs), interspersed with functional genes has allowed statistical analysis of the inheritance of those markers and disease phenotypes within families. Knowing the position of those markers and determining which of them cosegregate (i.e., are “linked”) with the disease in families has led to the “positional” mapping of >1,600 human disease loci. However, the gene vicinity (locus) is only identified to between 100 and 5,000 kbp, and there may be hundreds of genes within the region. Narrowing down to the culprit depends on short-listing candidate genes based on mechanistic knowledge of disease pathogenesis and/or examining each and every gene in turn until disease-causing variants are found.

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**Hypertrophic Cardiomyopathy (HCM):  
A Disease of Sarcomeres and Energy**

Hypertrophic cardiomyopathy is the archetypal example of positional cloning in cardiovascular disease. Its description

in 1958 by British pathologist Donald Teare as a rare “tumour of the heart” based on asymmetric left ventricular hypertrophy (LVH) and outflow tract obstruction (5) has ceded to its recognition as a heterogeneous, relatively common disorder with a prevalence of ~1:500 (6). Its significance is underscored by its identification as the most common cause of sudden cardiac death (SCD) in young adults and in athletes (7).

In 1989, HCM was mapped to chromosome 14 in a French-Canadian pedigree with autosomal dominant disease, and, thereafter, mutations in cardiac  $\beta$ -myosin heavy chain (MHC) were shown to cause HCM (8,9). Subsequent studies have revealed that HCM is more complex than anticipated, exhibiting genetic (multiple disease gene) and allelic (multiple mutation) heterogeneity, with as many as 9 sarcomeric genes and >400, predominantly missense, mutations described (10) (Table 1). That led to the proposition that HCM is a disease of the sarcomere (11,12). Despite that success, comprehensive screening detects sarcomeric mutations in only ~60% of HCM families. Some mutations may have been missed by the indirect sequencing techniques used, but failure to find a mutation in such a significant proportion clearly indicates that other novel HCM genes are yet to be found. The identification of missense mutations in muscle LIM protein (MLP) represents an example of such novel HCM disease genes (13). Muscle LIM protein has an increasingly large repertoire of functions, including cellular differentiation, growth, and cytoskeletal organization. Such nonsarcomeric disease genes will reveal additional hits in the HCM pathogenic pathway and will drive an improvement in our understanding of disease.

The insight that HCM arises from perturbations of the sarcomere provided unheralded opportunities to explore its

**Table 1****HCM and Phenotypically Similar Syndromes:  
Genes, Chromosomal Loci, Gene Product, and  
Mode of Inheritance**

Chromosomal Locus	Gene	Protein	Inheritance
1q32	<i>TNNI2</i>	Cardiac troponin T	AD
2q31	<i>TTN</i>	Titin	AD
3p21	<i>MYL3</i>	Ventricular essential myosin light chain	AD & AR(?)
7q36	<i>PRKAG2</i>	AMPK- $\gamma$ 2 subunit	AD
11p11	<i>MYBPC3</i>	Cardiac myosin-binding protein C	AD
11p15	<i>CSRP3</i>	Cardiac muscle LIM protein	AD
12q23–q24	<i>MYL2</i>	Ventricular regulatory myosin light chain	AD
14q12	<i>MYH7</i>	$\beta$ -myosin heavy chain	AD
15q14	<i>ACTC</i>	$\alpha$ -cardiac actin	AD
15q22	<i>TPM1</i>	$\alpha$ -tropomyosin	AD
19q13	<i>TNNI3</i>	Cardiac troponin 1	AD
Xq24	<i>LAMP2</i>	Lysosome-associated membrane protein 2	X-linked

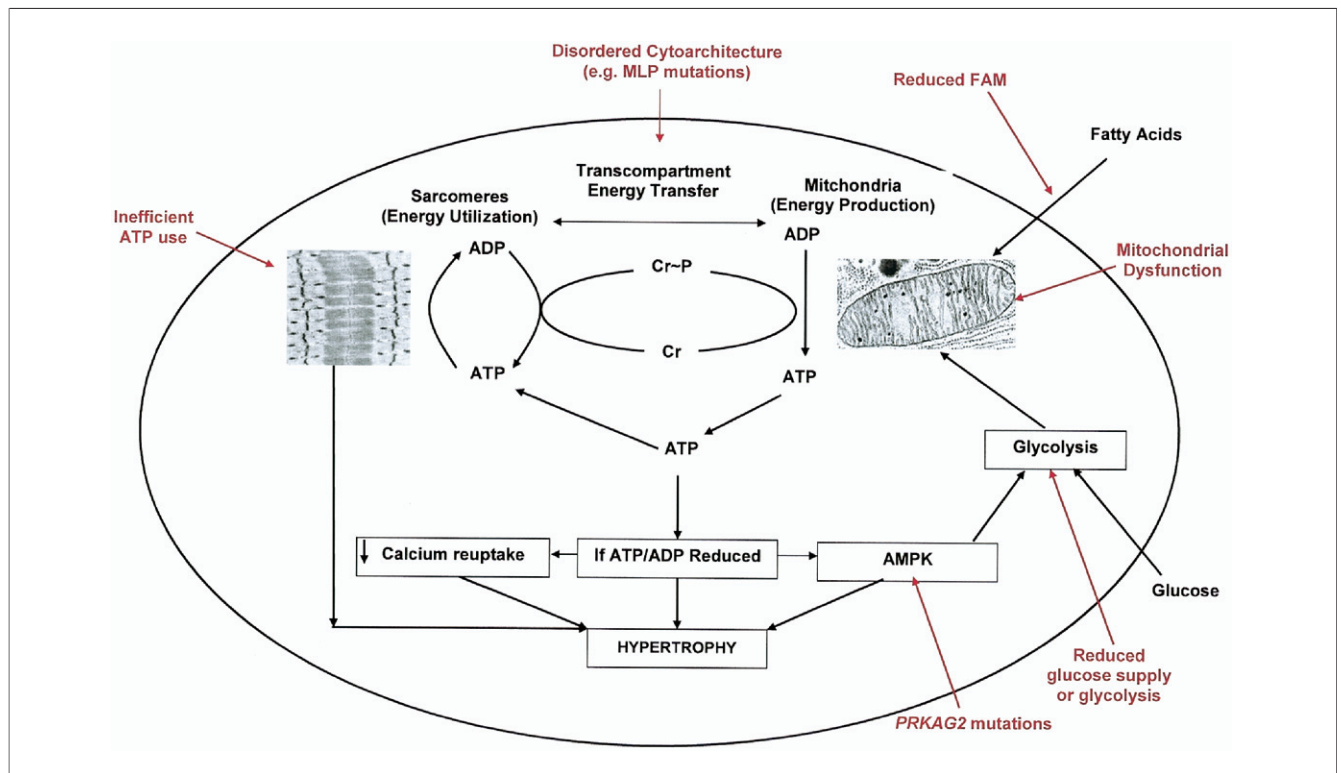
For citations to original references, see <http://genetics.med.harvard.edu/~seidman/cg3/index.html>.

AMPK = adenosine monophosphate-activated protein kinase; HCM = hypertrophic cardiomyopathy.

pathogenesis. The genetic findings led to the suggestion that mutated, putatively poorly functioning, sarcomeres may impair myocyte function, triggering adaptive mechanisms resulting in compensatory LVH (14). Although intuitively appealing, in vitro assays of sarcomeric function demonstrated divergent results: Although a few HCM mutant proteins reduced maximum force generation, the majority enhanced contractility (15). The “compensatory hypertrophy hypothesis” for HCM was accordingly refuted. These studies underscore the importance of validation of all genetic findings with translational genetic, molecular, and clinical studies.

Because no mechanistic knowledge of disease pathogenesis is necessary for successful genetic mapping, it has the merit of identifying unexpected genes and signaling pathways. Correspondingly, further genetic assessment identified mutations in the *PRKAG2* gene encoding the  $\gamma$ -2 subunit of adenosine monophosphate-activated protein kinase (AMPK) in a syndrome resembling HCM in conjunction with Wolff-Parkinson-White syndrome and progressive conduction disease (16). Adenosine monophosphate-activated protein kinase is a cellular energy sensor whose activation by energetic stress is one of the cell’s key mechanisms for energy regulation. Furthermore, disorders causing defects in

cardiac energy metabolism, such as mitochondrial mutations and Friedreich’s ataxia, exhibit HCM-like features. These observations gave weight to an emerging hypothesis, based on functional studies of mutant contractile proteins, that the unifying defect in HCM is energy deficiency. Sarcomere mutations are a potent source of energy deficiency through inefficient or profligate energy use (e.g., troponin T mutations) (17,18). The hypothesis has been supported by various clinical studies (19) and is increasingly accepted (20), extending the “disease of the sarcomere” concept to one of energy deficiency (Fig. 1). More recently, it has been recognized that even the missense mutations in MLP, which are only distantly related to sarcomeric function, can be integrated into this energy deficiency concept. Muscle LIM protein is critical to cytoskeletal architecture; the MLP-null mouse heart is a model of cytoarchitectural disorganization. It appears that a failure of energy transfer from its source of generation (mitochondria) to its site of use (sarcomeres) results in subcellular energy deficiency, contributing to energetic and contractile dysfunction (21). The combination of genetic linkage, functional genomics, and translational studies in HCM thus promises a rational approach to therapy, extending existing insights by identi-



**Figure 1** HCM: A Disease of Energy Deficiency

As indicated in red, the phenotype of hypertrophic cardiomyopathy (HCM) can arise from: 1) excessive energy use (e.g., by aberrant sarcomeres); 2) inadequate energy production (e.g., from poorly functioning mitochondria), inadequate metabolic substrates, or a failure to transfer energy across cellular compartments owing to cytoarchitectural defects as exemplified by muscle LIM protein (MLP) mutations; or 3) aberrant signaling of energy deficiency (e.g., with AMP-activated protein kinase [AMPK] mutations). The final common path for these diverse defects is energy deficiency and ensuing hypertrophy. ADP = adenosine diphosphate; AMP = adenosine monophosphate; ATP = adenosine triphosphate; Cr = creatine; FAM = fatty acid metabolism.

fying remaining HCM mutations and providing further clues to disease pathways and therapeutic targets.

### **Dilated Cardiomyopathy (DCM): The End Result of Diverse Pathways**

Insights into DCM, a common disorder of cardiac chamber dilatation and systolic impairment, have developed in an analogous, albeit circuitous, manner. Whereas HCM is principally a genetic disorder, the DCM phenotype can be caused by ischemia, infection, hypertension, pregnancy, alcohol, autoimmune disease, and of course genetic inheritance. Traditionally, geneticists attempt to purify a genetic substrate by stratifying patients; however, apart from a family history, in DCM cases there is often little to distinguish the genetic and acquired variants. Nevertheless, a concerted effort to validate pedigrees (using echocardiographic screening for asymptomatic left ventricular enlargement) has confirmed that 20% to 50% of idiopathic DCM cases have an inherited disease (22).

Linkage mapping to identify novel disease genes in DCM has been difficult, because of the underlying problems of multiple disease phenocopies, incomplete and often age-related penetrance, and poor survival in affected pedigrees. Therefore, a number of loci have been mapped which have yet to reveal the underlying disease gene (Table 2). Instead, DCM gene discovery was initially driven by candidate gene hypotheses. For example, it had been noted that DCM was a feature of Duchenne's (~80%) and Becker's (~10%) muscular dystrophies caused by mutations in the dystrophin gene and that some DCM cases segregated as X-linked traits without myopathy. It was hypothesized that dystrophin and other cytoskeletal proteins might underlie some cases of DCM, a hypothesis that was ultimately confirmed (23). This appreciation of DCM as a disorder of impaired cytoskeletal force transmission and mechanotransduction led initially to DCM's sobriquet of a disorder of the cytoskeleton and to identification of related genes, such as cardiac actin (24).

Further studies in families with DCM (sometimes with associated features such as sensorineural deafness, conduction defects, or skeletal myopathy) subsequently identified more diverse DCM genes (25) (Table 2). Of note, specific DCM mutations have been identified in 6 sarcomeric genes previously known to cause HCM. The DCM and HCM mutations in the same genes have fundamentally opposite properties, and each causes one or another cardiomyopathy without apparent overlap (26). Moreover, mutations in the nuclear envelope intermediate filament proteins, lamins A/C, identified in Emery-Dreifuss muscular dystrophy with DCM, modify nuclear signaling. Taken together, these observations show that diminished force generation and transmission and altered mechanotransduction and myocyte signaling can all cause DCM. Abnormalities of "downstream" pathways can also produce the same phenotype; these include mitochondrial mutations that compromise

energy production and mutations in adenosine triphosphate (ATP)-sensitive potassium channels and phospholamban that modify calcium signaling. Genetic insights in DCM have informed our understanding of its pathogenesis, much as they have for HCM, but have revealed a more diverse set of underlying processes (27).

Other cardiomyopathies are earlier in their nosology. Arrhythmogenic right ventricular cardiomyopathy (ARVC) is an uncommon disorder of cardiac muscle exhibiting progressive fibrofatty replacement of the right and, variably, left ventricle. Ventricular dilatation, arrhythmia, and SCD are key manifestations of ARVC. Positional cloning has identified 11 loci, of which the gene has been identified at 5, including plakoglobin, desmoplakin, plakophilin-2, desmoglein-2, and desmocollin-2, each encoding a component of the desmosome junction complex. Desmosomes anchor intermediate filaments to the cytoplasmic membranes in adjoining cells, thereby conferring mechanical strength. The consequence of desmosomal dysfunction is myocyte detachment and death, with ensuing inflammation and fibrofatty replacement providing the substrate for arrhythmia and ventricular dysfunction. Thus far, these genetic findings provide the taxonomic insight that ARVC appears to be a specific phenotypic response to disruption of desmosome function (28,29).

### **Is Genetic Screening for Diagnosis and Prognosis Feasible?**

It is apparent that although genetic studies have yielded and will continue to yield invaluable biologic clues, they have also uncovered considerable unanticipated complexity. Can the identified genes be used to identify disease carriers?

**Screening.** A key finding exposed by genetic analyses is that penetrance (the likelihood that a mutation carrier will have overt disease) is incomplete in all forms of HCM even in adulthood and can be as low as 50% for some mutations (12,15). A consequence of this is that familial disease was previously under-recognized. Indeed, 90% of patients presenting with HCM in adult life will have familial disease inherited from one or the other parent. A related aspect of HCM's complexity is its profound phenotypic heterogeneity, e.g., in relation to the distribution as well as extent of hypertrophy, the presence or absence of a gradient, or the extent of ECG abnormalities. Although history, clinical examination, and electrocardiography may contribute to the diagnosis of HCM, the main tool for diagnosis remains echocardiographic assessment of LVH. However, echocardiography is not without its challenges; apart from having to exclude other conditions exhibiting LVH, the distinction between pathologic hypertrophy (hypertensive hypertrophy), physiologic hypertrophy (athlete's heart), and HCM has proved to be especially demanding (30). Further, there are difficulties in defining the period for which screening is necessary. Conventionally, this might begin at 10 years to 12 years of age and continue annually into adulthood (~21



**Table 2** DCM- and ARVC-Causing Genes, Chromosomal Loci, Gene Product, Mode of Inheritance, and Associated Distinguishing Phenotypes

Chromosomal Locus	Gene	Protein	Inheritance	Phenotype/Associated Abnormality
DCM autosomal dominant inheritance				
1p1–q21	<i>LMNA</i>	Lamins A & C	AD	DCM + conduction system disease
1p1–q21	<i>LMNA</i>	Lamins A & C	AD	DCM + skeletal myopathy (AD Emery-Dreifuss or limb-girdle muscular dystrophies) ± conduction-system disease
1q32*	<i>TNNT2</i>	Cardiac troponin T	AD	Pure DCM
2q14–q22	?	?	AD	DCM + conduction system disease
2q31*	<i>TTN</i>	Titin	AD	Pure DCM
2q35	<i>DES</i>	Desmin	AD	Pure DCM
3p22–p25	?	?	AD	DCM + conduction system disease
5q33	<i>SGCD</i>	δ-sarcoglycan	AD	Pure DCM
6q12–q16	?	?	AD	Pure DCM
6q22	<i>PLN</i>	Phospholamban	AD	Pure DCM
6q23	?	?	AD	DCM + skeletal myopathy (limb-girdle muscular dystrophy) ± conduction-system disease
6q23–q24	<i>EYA4</i>	Eya4	AD	DCM + sensorineural deafness
9q13–q22	?	?	AD	Pure DCM
9q22–q31	?	?	AD	Pure DCM
10q22–q23	<i>VCL</i>	Metavinculin	AD	Pure DCM
11p11*	<i>MYBPC3</i>	Cardiac myosin-binding protein C	AD	Pure DCM
12p12.1	<i>ABCC9</i>	ATP-sensitive K channel	AD	Pure DCM
14q12*	<i>MYH7</i>	β-myosin heavy chain	AD	Pure DCM
15q14*	<i>ACTC</i>	α-cardiac actin	AD	Pure DCM
15q22*	<i>TPM1</i>	α-tropomyosin	AD	Pure DCM
DCM autosomal recessive inheritance				
4q12	<i>SCGB</i>	β-sarcoglycan	AR	Limb-girdle muscular dystrophy + severe DCM
6p24	<i>DSP</i>	Desmoplakin	AR	DCM + woolly hair and keratoderma
DCM X-linked inheritance				
Xp21	<i>DMD</i>	Dystrophin	X-linked	X-linked DCM, Duchenne & Becker muscular dystrophy
Xq28	<i>G4.5</i>	Tafazzin	X-linked	X-linked infantile DCM, Barth syndrome, hypertrophic DCM, endocardial fibroelastosis, and left ventricular noncompaction
Xq28	<i>EMD</i>	Emerin	X-linked	X-linked Emery-Dreifuss muscular dystrophy
ARVC autosomal dominant inheritance				
1q42–q43	<i>RyR2</i>	Cardiac ryanodine receptor	AD	ARVD2, catecholaminergic polymorphic ventricular tachycardia
2q32	?	?	AD	ARVD4
3p23	?	?	AD	ARVD5
6p24	<i>DSP</i>	Desmoplakin	AD	ARVD8
10p12–14	?	?	AD	ARVD6
10q22	?	?	AD	ARVD7, skeletal myopathy
12p11	<i>PkP2</i>	Plakophilin-2	AD	ARVD9
14q12–22	?	?	AD	ARVD3
14q23–24	?	?	AD	ARVD1
ARVC autosomal recessive inheritance				
17q21	<i>JUP</i>	Plakoglobin	AR	Naxos disease, palmoplantar keratosis, woolly hair
18q12.1–12.2	<i>DSG2</i>	Desmoglein-2	AD	ARVD10
18q12.1	<i>DSC2</i>	Desmocollin-2	AD	ARVD11

ARVC = arrhythmogenic right ventricular cardiomyopathy; DCM = dilated cardiomyopathy.

years of age). However, even though the majority of mutation carriers develop HCM by the time of physical maturity (~18 years of age), HCM may first manifest either in childhood or, alarmingly, in later adulthood (10). Late

onset is well recognized for *MYBPC3* mutations but is also seen with other mutations. The corollary of these findings is that patients can develop LVH, and with it a risk for SCD, after the age of 21 years, and although a pragmatic age

cut-off will identify most patients, it will have a significant false-negative rate. In short, at any given time point, any degree of left ventricular wall thickness, even normal thickness, may be consistent with HCM and increased cardiac risk (4).

Conversely, other cardiac disorders may present with echocardiographic features indistinguishable from HCM. Termed phenocopies, those conditions do not, in many cases, carry the same prognostic implications as HCM. They are well recognized in the pediatric setting and include Noonan syndrome resulting from *PTPN11*, *KRAS*, and *SOS* mutations, mitochondrial myopathies, glycogen storage diseases, and infiltrative myopathies. Distinguishing these conditions from HCM is often important and difficult.

To contend with this incomplete age-related penetrance and to increase ascertainment in the "at-risk" population using clinical tools, either echocardiographic screening would need to continue indefinitely (at great financial and emotional cost), or a more sensitive form of echocardiography (e.g., tissue Doppler imaging) would have to be validated that would identify patients with HCM but no overt LVH (31). It is also apparent that with respect to phenocopies, even these advanced techniques may be inadequate to diagnose or exclude HCM. With the identification of mutations in HCM, DCM, and ARVC, optimism has grown for affordable, rapid, sensitive, and specific genetic testing. For modest costs (~\$2,000 in the U.S., less in Europe) and <10 ml blood, patients or their physicians can now test directly for HCM mutations (10). Although this is one of the first direct clinical applications of genetics in cardiac disease, and has considerable utility in the right setting, it is not without limitations. The question remains: In what contexts can genetic screening be practicable?

**POPULATION SCREENING.** Although research studies have assessed the number of HCM gene carriers with increased left ventricular wall thickness in the community (32), this would not be feasible for routine clinical surveillance. First, the "pick-up rate" for mutations is 50% to 70% at best; therefore, a large number of false negatives would be generated in community screening. Secondly the effort to screen thousands of kilo-base pairs, even for only the 8 sarcomeric HCM genes, remains a significant exercise even with state-of-the art technology. One solution would be to restrict screening to *MYH7* and *MYBPC3*, which account for 80% of HCM mutations identified (33), but the false-negative rate would be higher and important classes of mutations (e.g., in cardiac troponin T) would be missed. Importantly, similar arguments apply to patients suspected of HCM; if the clinical likelihood is low, then the yield from genetic screening is too low for the analysis to be affordable. Moreover, a negative screen cannot rule out HCM.

**CASCADE SCREENING.** In contrast, genetic screening is valuable in evaluation of the families of index patients known to have HCM. There is a reasonable probability

(50% to 70%) that presenting patients will have their mutation identified, if the proband mutation is identified the rest of the family can be definitively and rapidly screened (at much lower cost). The Bayesian principle that in an autosomal dominant condition, parents, siblings, or offspring have a 50% pretest probability of being positive for a known mutation (rather than the 1 in 500 to 1 in 300 chance of being positive for any gene mutation in the population) makes genetic testing within families highly rewarding. This "cascade" screening of potentially affected family members avoids the problems of incomplete age-related penetrance and phenocopies. Unaffected individuals can be conclusively reassured and discharged, and the affected individuals within the extended family can be identified. In contrast, family screening with clinical tools tends to be ineffective because of false-negative results that terminate the cascade inappropriately. Cascade genetic screening of HCM families is already useful and practicable and provides a mandate for routine genotyping of newly diagnosed patients who have relatives at risk. Particular benefits apply in those families where clinical diagnosis is more difficult owing to incomplete penetrance, not least in troponin T mutations where risk of SCD is often high (see subsequent text).

Although genetic screening is not practicable for the majority of patients with DCM, assessment of patients with DCM associated with specific clinical features may be more rewarding. Genetic screening of patients with DCM as part of a skeletal myopathy syndrome, or with revealing features such as conduction defects, may be immediately clinically applicable. Up to 30% of the latter patients have been reported to carry lamins A/C mutations and therefore should be considered for screening. Similarly, the mutation yield in ARVC may soon be adequate for clinical utility. In all cases, negative genetic screens remain common and cannot rule out disease, but the value of a positive result for cascade screening in familial assessment is considerable.

**Prognosis.** While a number of clinical parameters are used to stratify HCM patients, individually they are of limited value. In HCM the most powerful predictor of sudden death is previous cardiac arrest (7-year mortality of ~67%). Even a personal history of syncope or a family history of SCD has positive predictive value (PPV) and negative predictive value (NPV) of ~25% and ~85%, respectively. Commonly used HCM risk factors, such as nonsustained ventricular tachycardia on Holter monitoring, hypotension on upright exercise, or severe LVH >30 mm, have low PPV (34). Over-reliance on risk factors with reasonable NPV but poor PPV mandates a need to better stratify patients, if only to reduce indiscriminate cardioverter-defibrillator implantation (35). Combining risk factors may allow global risk assessment with a better PPV; that approach, however, has not yet been prospectively evaluated.

An attractive alternative might be to institute genetic prognostication. However, the role of mutation analysis in the assessment of prognosis remains unclear. The first

obstacle is that all cardiomyopathy mutations are individually rare and many families will turn out to have a “private” mutation not previously described. Therefore, it will take time to build databases with sufficient cumulative mutation-specific data to give a reliable readout. The second obstacle is confounding through ascertainment bias. Mutations that consistently produce severe disease will be over-represented in families studied in specialist centers. Existing genotype-phenotype studies based mainly on large families ascertained in referral centers are therefore enriched for a subset of more highly deleterious genes; early studies tended to overestimate the prevalence of such variants. In contrast, most mutations produce a spectrum of disease severity, influenced by modifier genes and/or environmental factors. To reach a referral center, probands will have manifested a severe expression of HCM (e.g., outflow tract obstruction requiring intervention, threatened SCD, a family history of SCD, or severe symptoms). Asymptomatic (or mildly symptomatic) mutation-positive pedigree members tend not to present for assessment. Therefore, studies on series of individual probands are subject to even more significant referral bias and will overestimate the penetrance and severity of a gene or given mutation. An ideal study would comprehensively genotype a large population and systematically assess its phenotype longitudinally (36); that would avoid bias. However, the magnitude of this endeavor makes it unrealistic. A compromise would use probands to identify pedigrees, but after comprehensive cascade genotyping would exclude the index case from analysis, because the proband’s phenotype tends to overestimate disease severity (37).

The recognition that members of some HCM pedigrees were at increased risk of symptoms or SCD led to the recognition that individual mutations were associated with different prognoses (38). Thereafter, certain  $\beta$ MHC mutations were nominated as malignant: R403Q, R453C, G716R, R719W. Mutations in troponin T, accounting for ~5% to ~10% of HCM cases, are characterized by relatively mild and sometimes subclinical LVH but a high incidence of SCD (39). In contrast, other missense mutations in  $\beta$ MHC (N232S, G256E, F513C, V606M, R719Q, and L908V) and the troponin T S179F mutation were designated as benign. Even though specific mutations may be convincingly associated with a malignant or benign outlook, this should not be misinterpreted to imply that those variants will be found at an appreciable rate in patients with those clinical characteristics (all mutations are individually rare and there will be very large numbers of malignant and benign examples). Importantly, many studies indicate that there are many individual exceptions to such genotype-phenotype predictions, and that genotype is only one factor among many that determines individual outcomes (36,38,39). In addition, the coinheritance of compound mutations (more than 1 mutation in an individual HCM gene, or mutations in 2 HCM genes) is more common than might be expected and can explain why different families

that appear to have the same mutation can behave differently (40).

### Complex Trait Analysis: Can Modifier Genes Provide Therapeutic Insights?

Representing any disorder as purely monogenic is an oversimplification; even seemingly simple mendelian disorders show features that approximate to complex traits caused by the interactions of multiple genetic and environmental factors. This explains the inadequacy of existing approaches to fully explain the variability in cardiomyopathy phenotypes. Conversely, the offspring of patients with heart failure have a 2-fold increase in risk of ventricular systolic dysfunction; this association may be explained by the aggregation of genetically transmitted risk factors, such as hypertension and diabetes, and/or acquired factors that persist within families, such as culture and diet (41). Additionally, this familial predisposition raises the possibility that heart failure is a complex genetic trait that is in part determined by unidentified genetic influences. Linkage is often inadequate to detect such effects. Even if a locus has a moderately large phenotypic effect, linkage analysis is unlikely to be successful, even with large family datasets (>3,000 sib-pairs). Therefore, common alleles with a modest contribution to trait variance are best detected by association methods (42). The most common form of association study compares the frequency of sequence variants, e.g. SNPs, in a case-control study. A variant (allele) is said to be associated with the disease if it is overrepresented in case compared with control subjects.

**Genome-wide association studies.** A population acts as a large outbred pedigree with innumerable crosses; multiple meiotic recombination events have taken place, and therefore genetic markers that continue to be linked with a mutation are those that are in close physical proximity and in “linkage disequilibrium.” It is now recognized that the human genome (3.3 billion base-pairs) consists of 20,000 to 25,000 genes. More than 99.9% of the genome is conserved, and the remaining genome that constitutes the differences between individual human beings principally (>90%) comprises SNPs. Single-nucleotide polymorphisms are mutations occurring at single bases and may occur within or outside sequences coding for proteins or regulating their expression. In total, >7 million SNPs with a frequency of >5% exist, of which an assorted 3 million are passed on by a parent to an offspring. The human genome is not homogeneous but, according to population, comprises “haplotype” blocks of conserved DNA containing linked SNPs that are inherited en masse. By identifying a single or a few SNPs, the whole block can be deduced. Rapid automated array techniques can compare the frequency of such “tagging” SNPs (~500,000 distributed across the genome) between case and control subjects to systematically search for variants that contribute to the phenotype. Al-

though this approach has been used in coronary disease, it has yet to be exploited in cardiomyopathies.

**Candidate gene studies.** Candidate gene studies are in principle similar to genome-wide association studies, in that they compare the frequency of gene variants in case and control subjects. However instead of systematic studies across the whole genome, they concentrate their genetic power on a small number of SNPs that are suspected to be pertinent. They theoretically require fewer patients but are limited by the requirement for prior knowledge about disease pathogenesis to select gene variants. Although it has been reasonable to ascribe the HCM phenotype and prognosis principally to sarcomeric mutations, it is clear that there is extensive phenotypic variability in HCM not explained by single-gene defects. For example, in a large Scottish family with TNNT2-mutant HCM, 8 members died suddenly aged <30 years and 8 affected members survived into old age (39). The environment (e.g., hypertension) and modifier genes are therefore presumed to have a significant influence on HCM. Attempts to identify modifier genes in cardiomyopathy are therefore attractive, not least because modifier effects that protect mutation carriers from the disease phenotype may be an attractive target for intervention.

There are, however, many difficulties with candidate gene studies, some of which are generic and others relating specifically to aspects of cardiomyopathy. As many as 70% to 95% of association studies in cardiovascular disease fail to be confirmed, perhaps exacerbated by publication bias favoring an initial positive result (43). It is important to reduce spurious noise by investigating large, homogeneous, well phenotyped populations with carefully matched controls and to limit multiple hypothesis testing. Association studies may be confounded by ethnic stratification of the study population, requiring more sophisticated techniques for their resolution (42). Unfortunately, studies in HCM or DCM tend to be small and the populations heterogeneous. For example, modifier effects may differ with different underlying cardiomyopathy disease gene, or even mutations. One attractive study design for dealing with this is to analyze large founder effect families where all affected subjects share the same exact mutation. End points for modifier effects are also difficult to define and quantify; variation in hypertrophy in HCM in particular is not easily captured by any single, normally distributed, continuous variable. For example, maximum wall thickness and estimated cardiac mass will yield quite different findings in the same family.

Published association studies to test putative modifiers in cardiomyopathy need to be interpreted against this background. The angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism has been tested in HCM in several studies. It may be reasoned that because the DD genotype is associated with greater plasma ACE concentrations it would be associated with greater disease severity. A recent study concluded that polymorphisms in renin-

angiotensin-aldosterone system (RAAS) genes influence degree of LVH in 26 gene carriers from 1 family with an *MYBPC3*-HCM mutation (44). Similar studies have also been attempted in DCM, e.g., to evaluate functional variants in the  $\beta_1$ -,  $\beta_2$ -, and  $\beta_3$ -adrenergic receptor genes. A number of studies of varying size and quality have pursued this hypothesis and have again produced contrasting and inconsistent results with respect to both the direction and the magnitude of effect. Similar conclusions were drawn about RAAS polymorphisms (45) and those in adenosine monophosphate deaminase (AMPD-1) gene (46).

In conclusion, although the associations described are biologically plausible (inevitable, given the selection of the gene candidates), study design limitations so far preclude any lasting conclusions from being made. Genome-wide studies, with better study designs, have the potential to raise the reputation of association studies and potentially provide novel mechanistic and therapeutic insights. However, at present it remains unknown if adequate power can be achieved with the study sizes likely to be achievable in relatively uncommon disorders such as the cardiomyopathies. While human genetics struggles with these limitations, perhaps greater potential exists in systematic mapping strategies for modifier genes in rodent models of cardiomyopathy. Such approaches may have different limitations (chiefly, applicability to humans) but are at least tractable (47).

### Genomics of Cardiomyopathies: The Culmination of New Taxonomy?

In addition to the contribution of genetics to the reclassification of cardiomyopathies (4) and the mechanistic interest in the genetics underlying HCM/DCM, there is an aspiration that these studies will also reveal the mysteries of LVH, ventricular remodeling, dilation, and decompensation that result in heart failure. Although the concept of subtle variants in HCM/DCM genes combining to make small but cumulatively discernable contributions to common diseases is attractive (32), this appears to be unlikely. Using these “pure” diseases to dissect out mechanisms and pathologic pathways underlying common disease seems more plausible.

Although the expression patterns of many of the 20,000 to 25,000 genes of the genome are pertinent to disease progression, they are not captured by conventional genetic approaches that concentrate their power on small numbers of influential genes. An ideal assay would integrate the complex dynamic combination of genetic, epistatic, environmental, and epigenetic factors that, through modification of the cellular transcriptome, determine phenotype. DNA or RNA microarrays use “chips” to sample a large proportion of a cell’s mRNA transcriptome and to provide a snapshot view of gene expression (1). Arrays can be applied to the powerful study design of comparing 2 related biologic groups to identify differential gene expression. Once combined with complex analytic tools, including clustering algorithms



that identify genes that appear to be coregulated, arrays can identify transcriptional networks that are pertinent and indeed proximate to disease. Not only is this quantitative and qualitative dataset informative from a mechanistic perspective, but it can also identify transcriptional programs (“fingerprints”) amenable to therapeutic intervention and prognostically subclassify otherwise conventionally indivisible disease states (1).

Significant methodologic limitations remain. 1) The ultimate mediators of disease are not mRNA but their derived proteins; the transcriptome is not a perfect surrogate for the proteome with its added post-translational and compartmentalized complexity. Analysis of the transcriptome may fail to reveal the complexities of disease pathways. 2) Although the details of the array’s RNA signal analysis is beyond the scope of the present review, different laboratories use diverse approaches to “clean” their dataset. To ensure that the data from different laboratories is standardized, standards such as the “minimal information about a microarray experiment” have been established (48,49). 3) The power of arrays comes at a statistical price; performing multiple tests (e.g., 10,000 tests per chip) will inevitably result in many false positives. Traditional techniques of adjusting for multiple testing (e.g., Bonferroni adjustment) are not wholly appropriate in this context; novel statistical techniques are required to reduce false positives while not ruling out pertinent genes. 4) Technical issues notwithstanding, the success of any array study is limited by the quality of biologic samples employed. Improvements in the reproducibility of commercial chips has shifted the emphasis away from the chip per se to the biology of the samples. It is essential that the sample of interest and the control samples be large enough in number and phenotyped sufficiently carefully with respect to the timing and state of the organism’s, organ’s, or cell’s life cycle to be comparable and reproducible (50). 5) Array findings must be subject to assessment with independent techniques such as real-time polymerase chain reaction (PCR) that confirm differences in RNA expression. Some contend that even real-time PCR may not suffice and that more sophisticated pathway analysis may be necessary (49). And 6) associations should be mechanistically confirmed in animal models and human disease to confirm their true biologic relevance.

**Do functional studies in cardiomyopathies reveal mechanistic insights?** Although HCM, DCM, and RCM have traditionally been considered distinct disorders, the identification of mutations in the same sarcomeric genes in HCM and DCM indicates that there may be common themes underlying these disorders. Two formal possibilities exist: 1) Different mutations in the same sarcomeric genes causing HCM or DCM trigger the same pathway to different degrees, resulting in a phenotype continuum ranging from LVH to dilatation; or 2) these different mutations activate distinct programs that remodel the heart differently.

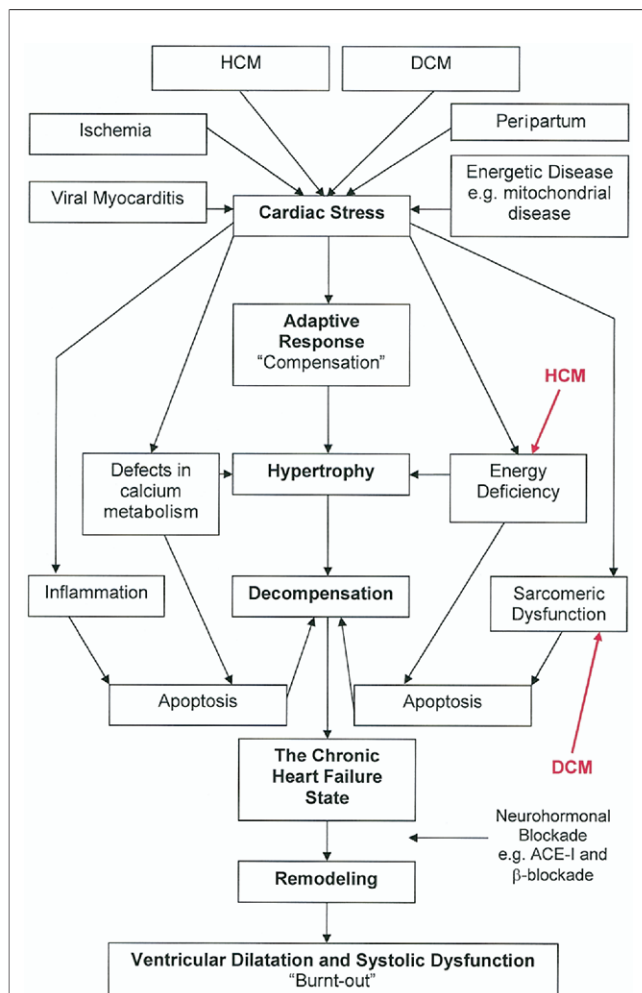
Although it is plausible that mutations causing differing amounts of mutant protein incorporation (51) or differently

mutated domains of the same protein could cause graded phenotypes, the experimental data support the distinct-program hypothesis. First, the histology characterizing HCM (i.e., myofiber disarray) is absent in DCM caused by sarcomeric mutations. Second, in vitro assays show that DCM and HCM mutations behave very differently. The DCM mutations depress myofibrillar function, whereas HCM-causing thin filament mutations enhance function (26,52). Specifically, the interrogation of 5 troponin T mutants, 2  $\alpha$ -tropomyosin mutants, and 1 troponin C mutant, all known to cause DCM, using in vitro ATPase, motility, and isometric tension generation assays demonstrates that, in contrast to HCM mutations, DCM mutants cause reduced  $\text{Ca}^{2+}$  sensitivity and reduced thin filament activation (26). The HCM and DCM mutations accordingly cause “distinct programmes.” Nevertheless, ~5% of HCM patients undergo dilatation characteristic of DCM (53), and mice bearing a truncation allele of *MYBPC3* show LVH in heterozygotes but DCM in homozygotes (54). How can this apparent gradedness be reconciled with the distinct-program hypothesis?

Apoptosis represents a critical pathway in the progression of heart failure, with myocyte attrition resulting in the remodeling characteristic of DCM (55). Apoptosis rates approach 0.08% to 0.25% of cells in patients with DCM compared with 0.001% to 0.002% in control subjects (56). Proapoptogenic animal models demonstrate that apoptosis contributes to cardiomyopathy; caspase inhibition prevents cardiac dilatation and improves left ventricular function (57). Although there is no biophysical relationship between DCM and HCM mutations that dilate, it is likely that DCM mutations stress myocytes more severely and trigger apoptosis (Fig. 2). In contrast, HCM myocytes “compensate” through hypertrophy; apoptosis is less prominent until decompensation supervenes. The similarity of the ultimate phenotype of dilated HCM to DCM thus results from late cellular rather than primary biophysical changes.

**Do genomic studies in cardiomyopathies confirm and reveal mechanistic insights?** The proposed role for apoptosis in HCM and DCM has been supported by a number of transgenic animal models, including the  $G_{q\alpha}$  model of LVH in mice. Moderate levels of  $G_{q\alpha}$  signaling induce LVH; more profound  $G_{q\alpha}$  activation results in myocyte apoptosis and DCM (57). That sequence of events was confirmed with array technology in differing rat models of pathologic LVH and RVH; the transition between hypertrophy and dilatation corresponded to the activation of apoptosis (58,59). Genomic studies in animals are thus consistent with the hypothesis that DCM is a nonspecific phenotype, with severely stressed myocardium approaching a “burnt-out” proapoptotic state where the rate of apoptosis exceeds cardiac compensation. In contrast, LVH represents a more complex state, where compensation occurs through increases in cell size and, probably, number.

Human genomic studies have so far proved to be less informative. Methodologic challenges are posed by compar-



**Figure 2** The Mechanisms Through Which Cardiac Stresses Incite Ventricular Dilatation Dysfunction

Diverse cardiac stresses, either through direct toxicity to myocytes or through processes such as inflammation and/or aberrant calcium dynamics, result in myocyte apoptosis. The heart initially instigates compensatory responses according to the nature and severity of the inciting influence. If energy deficiency is a prominent feature of the inciting stress, then hypertrophy may initially ensue. If compensatory adaptation through hypertrophy and perhaps stem cell recruitment is inadequate, unchallenged apoptosis will result in myocyte depletion and myocardial decompensation. The resulting low cardiac output state promotes an initially adaptive but a chronically maladaptive neurohormonal state that perpetuates myocyte apoptosis. This common maladaptive chronic heart failure state is, at least in part, independent of the proximate inciting influence and is characterized by neurohormonal activation, metabolic impairment, and maladaptive cardiac remodeling that results in dilated-hypokinetic ventricles. ACE-I = angiotensin-converting enzyme inhibition; DCM = dilated cardiomyopathy; HCM = hypertrophic cardiomyopathy.

ing genomic snapshots derived from differing parts of the heart and from patients at different stages of heart failure with varying therapeutic regimens (ranging from medical therapy to left ventricular assist device [LVAD]-rescued hearts). Patient samples often differ with respect to age, gender, and ethnicity. Further, the choice of controls may be limited (60). These heterogeneities, sometimes coupled with small patient numbers, and the technical limitations

already described limit the robustness of human datasets. Nevertheless, without resorting to the intuitively appealing concept of serial myocardial sampling of a homogeneous self-controlled cohort of patients (61), a number of investigators have succeeded in identifying transcriptomes characteristic of distinct cardiomyopathies (62). Although those findings contribute phenomenologically to the new taxonomy of cardiomyopathies and may also discern mechanistic pathways, it cannot be overemphasized that they represent statistical association, not causation. The latter proviso was recently exemplified by a comparison between failing and LVAD-rescued hearts. LVADs have, in some cases, induced regression of cardiac dysfunction (63). However, similar transcriptomes have been identified in both the failing and LVAD-unloaded hearts. To have assumed that the heart failure transcriptome was causative of only the dilated state would have been erroneous and potentially misleading (64).

Notwithstanding these methodologic provisos, human studies have confirmed the distinct nature of HCM and DCM (65). They have also identified a proapoptotic shift in tumor necrosis factor- $\alpha$  signaling as a potential target for the transition from LVH to decompensated dilatation (66). Genomic profiling has supported genetic taxonomic studies by distinguishing between different forms of cardiomyopathy, including DCM (67), ischemic (68), alcohol (69), HCM (65), and Chagas' disease (70) cardiomyopathy. Although by their very nature a comprehensive list of genomic changes would be lengthy, altered energy metabolism appears to be one of the most consistent features of different cardiomyopathies (60). These include changes in mitochondrial, glycolytic, and lipolytic transcriptional profiles (60). Recently, a careful genomic comparison of 2 transgenic mouse models of HCM that express different mutations in the same gene ( $\alpha$ -tropomyosin) confirms that energetics appears to be a key phenotypic determinant of HCM (71). Contemporaneous molecular studies have confirmed the role of energy compromise due to excessive ATP consumption in HCM (72). Even genomic studies comparing heart transplant recipients with patients suffering from *Trypanosoma cruzi* cardiac infection, suggest that energetic disease is a proximate cause for heart failure (73).

### Genomics of Cardiomyopathies: Implications for New Therapies?

Therapies for cardiomyopathies have been based on morphologic similarities with the more common acquired heart muscle diseases. This approach has been successful partly because of the nonspecific nature of existing medical therapies and partly because of the limited physiologic repertoire of responses to cardiac stresses. Nowhere is this more evident than in DCM. Transcriptome analyses have supported the view that a dilated ventricle represents a common "burnt-out" phenotype from almost any cardiac insult severe enough to preclude compensation; the resulting chronic

heart failure state has been regarded as independent of inciting etiology and much more a reflection of detrimental systemic neurohormonal activation (74). In DCM this has been supported by consistent measurements of neurohormones and vindicated by the corresponding success of treatments such as ACE inhibition, beta-blockers, and cardiac resynchronization therapy. Even in DCM, however, the variability recognized in transcriptome analyses between alcoholic, ischemic, and inherited cardiomyopathies has reawakened interest in a tailored approach to novel therapies. The recognition that metabolic changes represent a critical feature of DCM and that glucose metabolism is down-regulated and fatty acid metabolism is up-regulated (75) has led authors to propose therapeutic agents “which shift myocardial cells from fatty acid to glucose metabolism” (76). Metabolic modifiers are being actively investigated in DCM; a recent study demonstrated that perhexiline, a carnitine palmitoyltransferase-1 inhibitor, through augmenting glucose and blocking fatty acid metabolism, resulted in unprecedented changes in cardiac function (77). This approach was successful in both ischemic heart failure and inherited DCM, underscoring similarities in final common path. Based on array studies, similar processes of energy deficiency may also be pivotal in Chagas’ and transplant cardiomyopathy (70,73).

Consistent with the more specific genetics and transcriptome of HCM, it has proved to be more challenging to draw therapeutic parallels between genetic and acquired variants of LVH (e.g., HCM vs. hypertensive LVH). There is little evidence to suggest that RAAS blockade has any role in HCM. Moreover, treatments successfully applied to HCM, such as cardioverter-defibrillator implantation to prevent SCD, beta-blockers, and septal ablation, do not treat the underlying disease (78). Even therapeutic modalities based on disease physiology are often only minimally successful. Dual-chamber pacing has been reported to reduce left ventricular outflow tract gradients, but although pacing appears to be relatively successful in controlling symptoms in selected patients (79) randomized trials do not support a major role for pacemaker implantation (80). Transcriptome analysis has the capacity to guide therapy; there is extensive evidence that metabolism also plays a role in the different forms of LVH. Evidence from array analyses indicates that the distinction between physiologic and pathologic hypertrophy includes differences in glucose and fatty acid metabolism (81); this provides a potential new metabolic therapeutic node in cardiac hypertrophy.

As noted in the foregoing, HCM represents a prototypical disease. Insights from genetics, biophysical studies, and genomic analyses have been combined with careful clinical phenotyping and proof-of-principle physiologic studies to implicate a causative rather than a correlative association between compromised energetics and HCM. Trials are underway to assess the capacity of myocardial metabolism to modify HCM progression. If disease-modifying treatment does become available it is likely to be more effective in

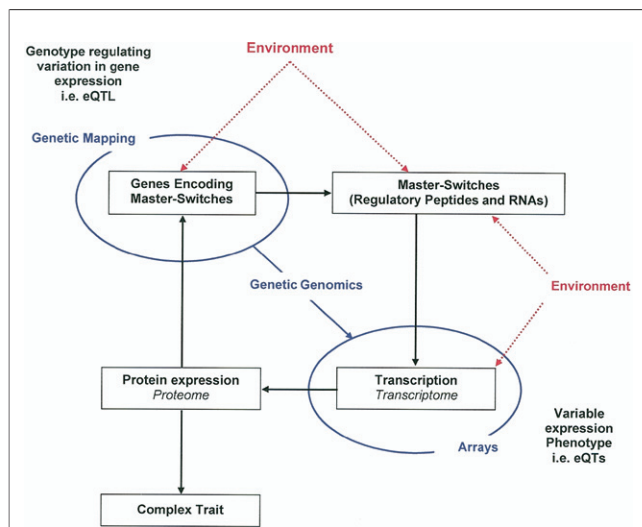
early, perhaps preclinical, disease. In that situation, systematic genetic analysis and cascade screening for identification of HCM-gene carriers will be mandated, with at last a real chance to reduce HCM morbidity and mortality.

### Genomics of Cardiomyopathies: The Future?

There is good reason to be optimistic about the continuing influence of new genetic findings on our understanding of cardiomyopathies. Even very rare mendelian disorders can reveal new insights with far-reaching consequences, and the cardiomyopathy disease genes remaining to be discovered are likely to be in unexpected genes, implicating new pathways. Progress in identification of susceptibility genes and modifier genes should follow improvements in automated technology and growing experience with complex data sets, including genome-wide association studies. Similarly, great optimism pertains to further developments in functional genomics. Genomic tools will have the capacity to systematically and rapidly screen a global genomic repertoire, e.g., of transcripts, proteins, or metabolites, and to marshal findings using cluster analysis. However, these tools are simply descriptors without the power of genetics (based on the principles of cosegregation) to determine causality. The combined power of both genetic and genomic approaches is therefore attractive as illustrated by the early progress with “genetic genomics.” In that approach, measurements from genomic analyses (e.g., transcript levels) are analyzed genetically as quantitative traits. In this way, causal associations between gene variation, gene expression, and disease are defined.

The recognition that the human genome contains as few as 20,000 to 25,000 genes has been surprising. This surprise has been reconciled by the appreciation that much of the complexity of our physiology is determined not by large differences in our genetic hardware, but by how complex transcriptional programs, guided by transcriptional master-switches (genetic software), manipulate the smaller gene repertoire. Thus, phenotypes are determined by transcriptional profiles. Genes that are expressed as part of a master-program may be determined by adjacent variants on the same stretch of DNA (*cis* regulation) or by signals from remote regions (*trans* variants). The patterns and magnitude of these expression patterns are variable among individuals (thus forming “intermediate phenotypes”). That variability, in turn, is attributable to inherited influences (polymorphisms that act as “expression quantitative trait loci”) in master-switches. Taking these factors into account, it is possible to identify which loci are implicated and the extent of their contribution to disease expression through linkage studies (82) (Fig. 3). Genetic genomics provides the opportunity to triangulate onto these master-switches and therefore to identify the ultimate determinants of complex traits. The loci determined by these studies can then be corroborated by mapping of traditional phenotypic traits and by functional studies. This has already been achieved in murine





**Figure 3** The Rationale of Genetic Genomics

The ultimate determinant of biologic phenotype is the pattern of cellular protein expression (i.e., the proteome). The proteome is in turn determined largely by transcription within the cell (i.e., the transcriptome). Microarray technology permits a comprehensive and quantitative description of the transcriptome. Traditional approaches to understanding disease pathogenesis have attempted to correlate genetic variants with gross phenotype. Instead, genetic correlations can be made with messenger RNA expression patterns as measured by arrays. These intermediate phenotypes are termed "expression quantitative traits" (eQTLs). The combination of the systematic power of arrays to measure transcription and the power of genetic analysis to identify causality has proved to be powerful. The genetic determinants of gene expression, termed "expression quantitative trait loci" (eQTL), represent *cis* and *trans* regulatory elements in which variations cause alterations in cells' gene expression patterns. Identification of variants in these regulatory elements, termed "master-switches," not only identifies key molecular protagonists in health and disease *per se*, but also implicates downstream pathways with therapeutic implications.

disease models but is some way off in humans. If these approaches can be made to work in human populations, and if (as will be the case for at least some biologic systems) RNA from peripheral blood will be informative (83), genetics and genomics will together accelerate progress in human disease.

**Reprint requests and correspondence:** Prof. Hugh Watkins, Department of Cardiovascular Medicine, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU, Oxfordshire, United Kingdom. E-mail: hugh.watkins@cardiov.ox.ac.uk.

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